

Experiment 10: Conduction Velocity

OVERVIEW

In this lab you will:

- Learn how to measure the speed of action potentials using a new earthworm preparation
- Learn about the difference between sparse coding and rate coding
- Learn how to do multiple recordings in an animal

OBJECTIVES

Before doing this lab you should:

- Review the concept of conduction velocity
- Study the anatomy of the earthworm

After doing this lab you should be able to:

- Be able to design an experiment measuring conduction velocity in different earthworm preparations under different conditions
- Understand the two ways that animals increase their conduction velocity

MATERIALS

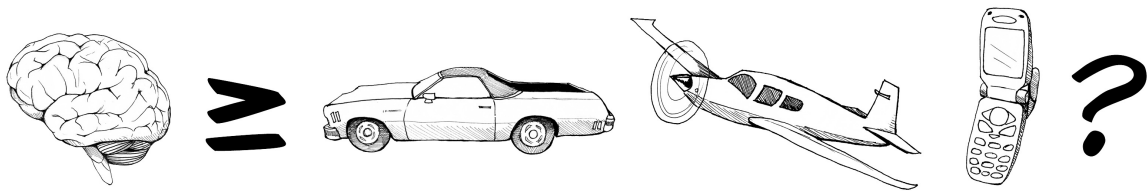
- 2-Channel SpikerBox
- Earthworm
- A Faraday Cage
- Laptop with stereo line-input
- Patch/Laptop Cable
- Ruler
- Balsa Wood to place earthworm on and insert electrode pins into

How fast are your neurons?

Background

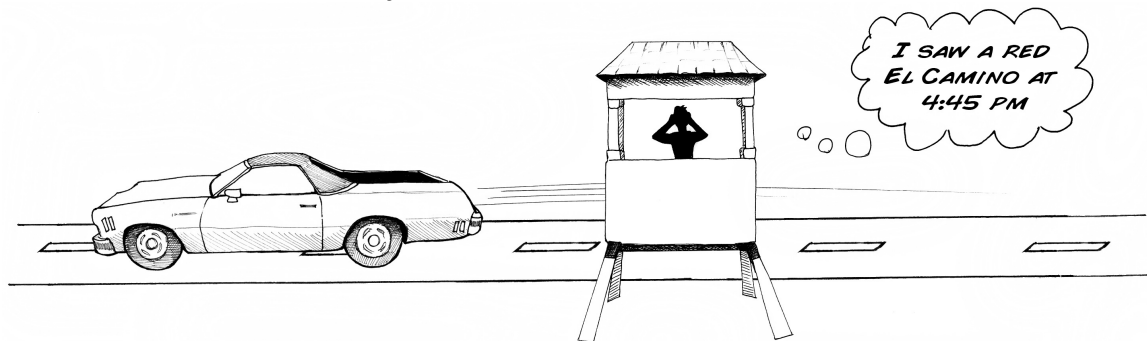
Up to this point we have been studying spikes emitted from crickets and cockroaches, mostly by monitoring the “spike rate” and “spike presence” in response to certain stimuli or conditions. We now will study “spike speed.”

You probably think the nervous system is pretty fast. You seem to hear the spikes immediately when you touch the leg of the cockroach or blow on the cerci of the crickets. But is it instantaneous? Of course not! Not even light, the fastest signal in the universe, travels instantaneously. But how fast is a nervous system? Is it faster than a car, faster than a jetplane, or faster than a cell phone? And how can we measure it?



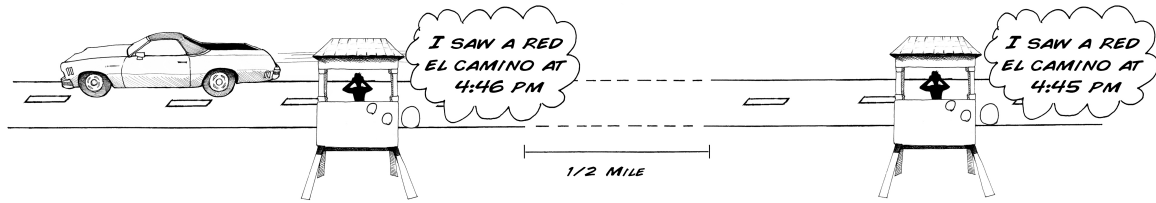
In all previous experiments, we’ve only recorded our neurons using one channel (meaning we use only one recording electrode and one ground). To measure speed (velocity) though, you need to measure both time (when a spike occurred) and distance (how far a spike has travelled down a nerve).

Take the analogy of a car on a highway. If you were looking out of a small observation hut by the road, you could tell what whether you saw a car, what kind of car it was, and the time that you saw it.



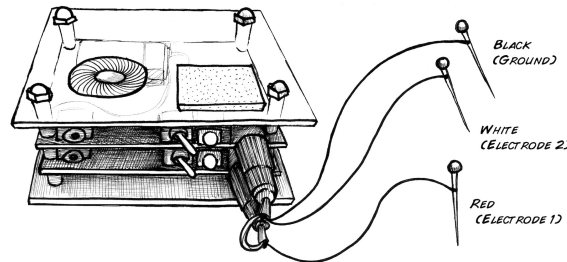
Similarly with your SpikerBox, you can currently tell if you saw a spike, perhaps what kind of neuron generated that spike (we will discuss this in a later experiment), and the time you saw the spike, but you can’t tell how fast the spike was travelling down the nerve.

Let’s go back to the car on the highway. Suppose you had a friend ½ mile down the road in a similar hut:



Later, you two could compare notes to determine the speed of the car. 1 minute = 0.016 hours. Dividing $\frac{1}{2}$ mile by 0.016 hours = 31.25 mph.

Thus, we can measure the speed with two observers, and that's why we use the "2-Channel SpikerBox" to measure two points along a nerve as a spike travels down it.



So, why don't we take our two-channel SpikerBox with our two electrodes and ground, put it in the cockroach, and measure the spike output of the two channels? You will notice immediately that there are a lot of spikes happening, in fact, way too many to keep track of it all.

Let's go back to our analog of the road. Imagine a very busy, fast moving street with many similar looking cars, say, Lakeshore Drive in Chicago, and you and your friend can only set up stations very close to each other.

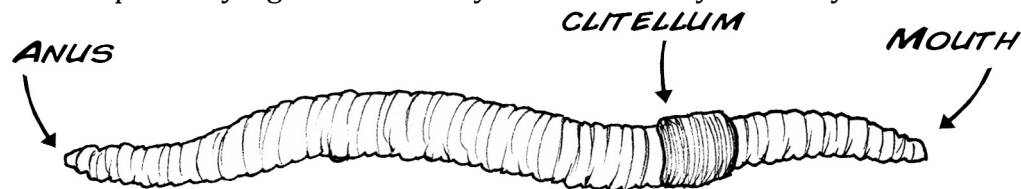


You can see the problem, there are a lot of spikes occurring in the cockroach leg, and identifying unique ones with two observers is very tricky. The femur of cockroach leg has 2 nerves inside, and inside each nerve are about 100-200 neurons, all firing

many spikes. We are also limited by how far we can place our electrodes from each other in the cockroach leg, as the leg is only about 8 mm long.

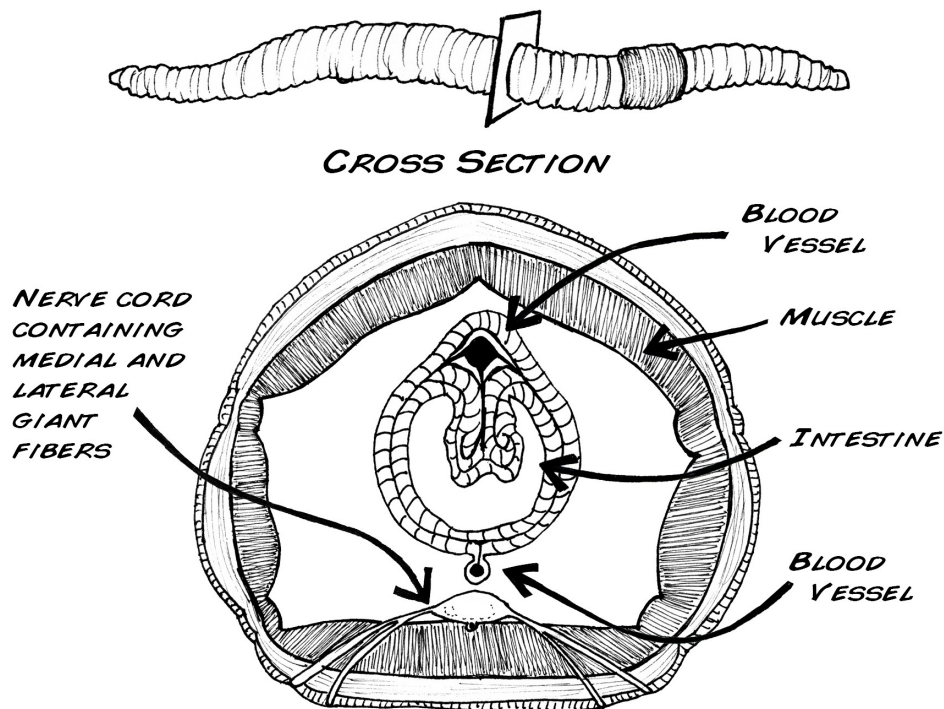
Ideally, given our limited tools, we'd want to measure spikes on a longer nerve, a nerve with only 1-3 large axons in it, and axons that do not fire many spikes.

Is there any creature in the animal kingdom that meets these qualifications? Yes! and it is probably right now under your feet and in your backyard.



TOP (DORSAL) SIDE OF WORM DARKER THAN BOTTOM (VENTRAL)

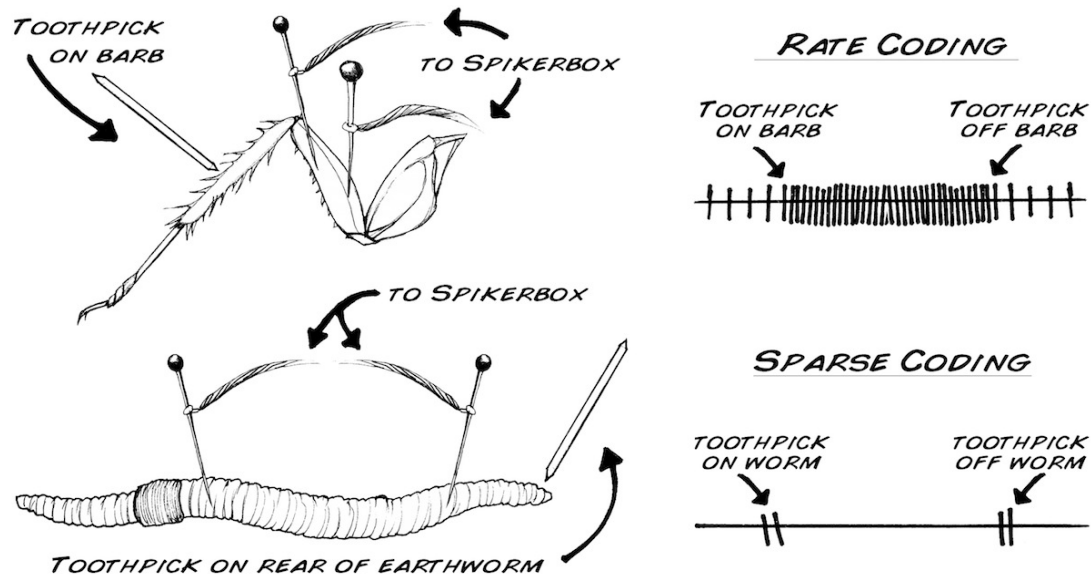
We have been studying arthropods (insects), but we now move to a new class of invertebrate: annelids! Or more commonly, worms! Enter our newest preparation: the common earthworm, *Lumbricus terrestris*. It's a simpler animal than what we've studied before, and the earthworm contains three large axons that run its length, the "medial giant" and the two "lateral giant" fibers. The medial giant fiber transmits information about the front of the worm (the part closest to the clitellum), and the lateral giant fiber transmits information from the skin cells of the posterior end of the worm (Kladt et. al 2010).



In addition to the earthworm's long length, which allows us to place our recording electrodes far apart, the earthworm also exhibits what is known as

Sparse Coding

What is sparse coding? Let's turn back to our cockroach and the "rate coding" you have previously studied. In rate coding, the intensity of a stimulus is encoded by the rate of spikes. If the cockroach leg used a sparse coding scheme, the leg nerves would only fire 1-2 times when you touched the barb with a toothpick, and 1-2 times more when you removed the toothpick.



This sparse coding scheme is what we will see in the earthworm experiment below, and we will exploit it to measure the conduction velocity (or speed) or the spikes.

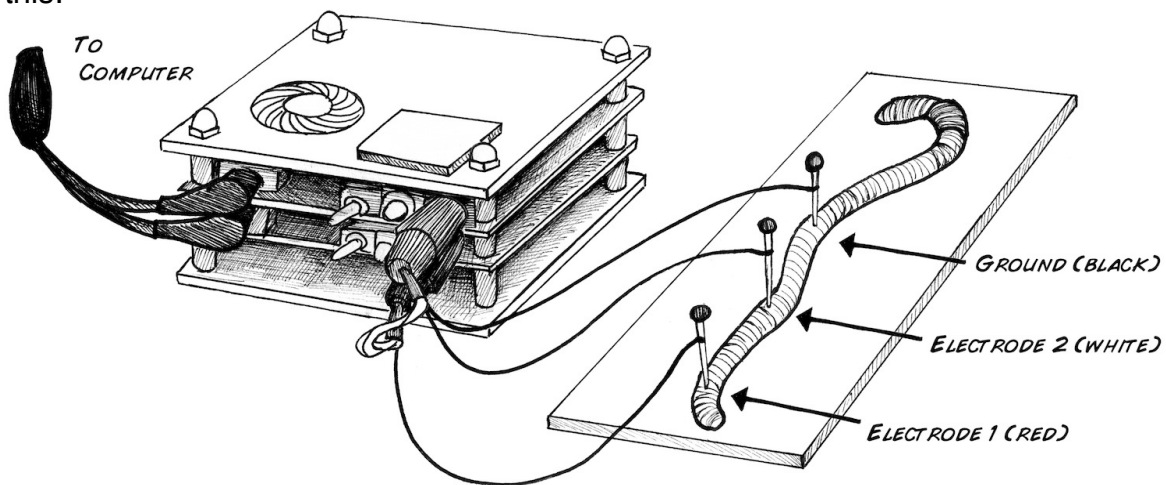
Procedure:

1. Go to your nearest pet store, sporting goods store, or gas station and purchase a box of earthworms (they are typically used to feed lizards, turtles, and fish. Fishermen use them as bait). They should be around \$3-\$4 for 12 worms. The Earthworm box should stay in the refrigerator (not the freezer) when not being used. The worms can last approximately 1-2 months.

2. Prepare a 10% ethanol solution. The easiest way to do this is to use vodka (which is normally 80 proof, or 40% ethanol). Since vodka is not much more than watered down pure ethanol, dilute it further to 1 part vodka, 3 parts water. For example, we mix 10 milliliters of alcohol with 30 milliliters of tap water. Ask your teacher to prepare this for you

3. Place a healthy earthworm in the alcohol mixture and wait seven minutes. Do not wait too long; as with human anesthesia, the delicate balance between too little anesthesia and too much is tricky. Too little anesthesia and the earthworm will move around during the experiment; the resulting muscle electrical activity (electromyogram) will drown out the small neural electrical signals you are interested in. Too much anesthesia and the nerves will not fire. We've found 7-10 minutes is a good range.

4. Place the Earthworm on a piece of balsawood or thick cork, and put your three electrodes of your two-channel SpikerBox in the posterior end of the worm, like this:



5. Place a Faraday Cage around the Earthworm, and clip the Faraday cage to the ground of either channel 1 or channel 2 of your SpikerBox (see our Faraday Cage write-up on wiki.backyardbrains.com for how to do this).

6. Turn on your 2-Channel SpikerBox, plug in your laptop patch cable to both the 2-channel SpikerBox and your Laptop, and Start the Program Audacity. Make sure your laptop line-in is capable of recording two channels. See our write-up on "testing stereo input" on wiki.backyardbrains.com for how to test this.

7. Make sure in the Audio I/O Preferences of Audacity to set your “recording device to “built-in input” for input and “Channels” to 2. Make sure “playback” is set to “Built-in Output”, and also check the “Hardware Playthrough” and “Software Playthrough” checkboxes so you can hear the spikes as you record them.

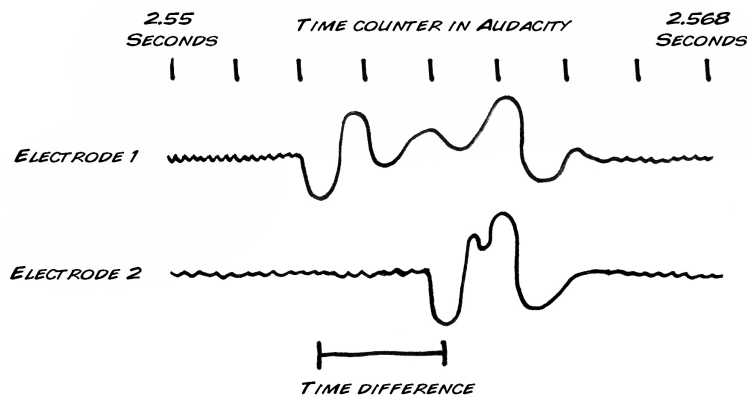
8. Press the Record Button in Audacity. You should hear some background noise, and now touch the anterior (rear) end of the earthworm with a toothpick. You should hear 1-2 faint “pops”. Those are the spikes. Interestingly, the neurons in the earthworm are myelinated (covered in insulating fat), and you will notice the spikes are much quieter than you are used to (Hartline and Coleman 2007). Many nerve diseases, such as Multiple sclerosis, are caused by a degeneration of this fatty covering.

9. Stop your Recording. Now, go back through your recording file and try to find your Spikes. You will have to zoom in multiple times to “stretch out” the signal enough to take a measurement. You should see the beginning of the spikes separated in time.

10. Using the time-axis, measure out how far apart in time the beginnings of the spikes are from each other.

11. Using a ruler with divisions in the mm range, measure the distance between electrode one and electrode two.

12. Divide the distance by the time. Viola! You have just measured conduction velocity.



Now start exploring. For example, does this measurement change from spike to spike? Does it change from earthworm to earthworm? Are smaller earthworms faster or slower than large earthworms. Is this speed sensitive to depth of anesthesia? These are all questions we would like to know, and you do to! Let us know what new discoveries you make.

Troubleshooting:

1 – If your earthworm is not healthy (not moving around the soil and not resisting/squirming when you try to pick it up), you will not get good recordings.

2 – For reasons our electrical engineering team has not been able to answer, the earthworm is a terrific antenna for electrical noise. Unless you are doing your experiment outside, you need to use a Faraday Cage for this experiment to work.

3 – You also need a laptop with a stereo (2- channel) line-input. Most laptops do, but some computers, like MacBook Airs and early MacBook Pro's, don't. Really the only to find out besides calling the laptop designers is to run a test we have designed. If you do not have a stereo line-in, you can use a USB mixer to get stereo analog inputs into your computer. We recommend the \$30 "iMic". Note that this USB interface causes a slight 1000 Hz "ring" in your recordings, but you can still successfully record earthworm spikes with this device, and sometimes you can ground the 1000 Hz ring out.

Diving Deeper

There are two ways that animals typically increase conduction velocity. In you and I, our nerves are covered with a fatty substance called myelin. In our friends the crickets and cockroaches, the neurons have larger diameters. Obvious, having large neurons limits the amount of neurons you can pack into a small space. The relationship between axon diameter and conduction speed is a function of the square root of the diameter. That is, if an axon is four times as large in diameter, the conduction velocity twice as fast. If the axon is nine times as large, the conduction velocity is three times faster.

Myelin works by decreasing the capacitance of the axonal, which decreases the time constant for recharging the membrane. This can become difficult to understand, we recommend Hartline and Coleman (2007) as a good starting reference.

Oddly, some creatures, like the Penaeidae shrimp and the earthworm you are studying today, have both large diameter axons and myelination. In the Penaeidae shrimp, the conduction velocity has been measured at 210 m/s, or 470 mph! But in the earthworm, we measured it at ~7.5 m/s, or 16 mph. Why is there such a difference in speed?

References:

Hartline and Coleman (2007) Rapid Conduction and the Evolution Review of Giant Axons and Myelinated Fibers. *Current Biology* 17, R29–R35

Kladt N, Hanslik U, Heinzel H-G (2010) Teaching basic neurophysiology using intact earthworms. *The Journal of Undergraduate Neuroscience Education* (JUNE), Fall 2010, 9(1):A20-A35

DISCUSSION QUESTIONS

1. We are we using alcohol to anesthetize the earthworm instead of ice water?

2. What happens if you reverse the ground and recording electrode 1?

3. What happens if you touch the anterior part of the worm (the mouth).

4. What are some advantages and disadvantages of sparse coding vs. rate coding?
